Chapter 13

Effect of Supercritical Fluid Extraction on Residual Meals and Protein Functionality

Fabio Favati[a], Jerry W. King[b], and Gary R. List[b]

[a]Dipartimento di Biologia DBAF, Università della Basilicata, Via N. Sauro 85, 85100 Italy; [b]Food Quality Safety Research Unit, National Center for Agriculture Utilization Research, ARS, USDA, 1815 North University Street, Peoria, IL 61604 USA.

Introduction

Over 80 years ago Bridgeman (1) reported that pressure (700 MPa) promotes the coagulation of egg albumin, an effect that Grant (2) later showed to result from cleavage of sulfhydryl groups in the protein. Early studies also showed that tobacco mosaic virus is denatured by pressure (3); several other papers published in the 1940s and 1950s noted the general effects of pressure on protein and enzymes (4, 5). However, aside from these early reports, the effect of pressure combined with a supercritical fluid did not receive much attention until the last decade, when several groups showed that supercritical carbon dioxide (SC-CO_2) could be used to recover lipids and oils from a variety of substrates (6–12). Other commodities processed by supercritical fluid extraction (SFE) during the 1970s and 1980s included coffee, hops, cocoa, and tea (13–15).

It is probably fair to say that the primary goal in many of the cited applications of supercritical fluid media is to produce an extract that is superior or equivalent to that obtained from a different processing or extraction procedure. Generally the effect on the material remaining in the vessel, after the extraction ends, is of secondary importance. Investigations to examine the extracted residuals have been motivated by curiosity as well as economic considerations. Certainly the presence of a proteinaceous meal that is solvent-free presents some interesting opportunities for incorporation into the animal and human food chain.

This chapter is primarily concerned with the effect of supercritical fluid processing on residual protein-based meals and their functionality in an anticipated end use. Unlike most of the chapters in this monograph, we have tried to focus on what is left over after the extraction rather than on the extract. Of course, extraction conditions and sample preparation have been noted, particularly when lipid removal is crucial to the production of the desired end product.

We have specifically avoided any coverage on enzymatic reactions in supercritical fluid media, because that is covered in Chapter 15. Likewise, the relative new area of ultrahigh-pressure food processing is outside the scope of this review. Fundamental studies on the effect of pressure on proteins have been noted where relevant, but that subject is normally in the realm of biophysical chemistry. What follows here, then, is a highly focused review describing what is known about natural-product residuals

remaining after supercritical fluid extraction, their proximate properties, and applicability to dietary requirements in man and animal, and their organoleptic properties and morphological alteration, which could play a role in food compounding.

Functional Properties of Proteins

Nowadays consumers are more discriminating about the quality of food items found on the market, and the success of several food products is strictly related to the satisfaction of the consumers' expectancies about the perceived quality, represented by attributes such as flavor, color, odor, and texture. The food industry is therefore increasingly looking for ingredients that have versatile but consistent functional properties and are also compatible with product formulation.

Besides their nutritive value, proteins constitute one of the most important classes of ingredients, because they can be incorporated in a variety of food items and have wide ranges of functional properties. Some of the most important functional properties of proteins are solubility, emulsifying properties, foaming properties, fat absorption and water absorption.

Solubility is of extreme importance for use of proteins in most food systems. Furthermore, several other functional properties (e.g. gelation, emulsifying and foaming properties) are strictly related to an initial protein solubilization step. Protein solubility is influenced by many factors, of which the most important are pH and ionic strength value. Plots of protein solubility data as a function of these two variables can give some information on the extent of the denaturation processes that could have occurred during previous technological treatments or processes, and that information can also be used to predict the kinds of food or beverage into which the proteinaceous material could be incorporated.

The emulsifying properties of proteins are related to their capacity to form and stabilize emulsions in food systems. Emulsion capacity represents the amount of oil that a protein solution or suspension can emulsify before the emulsion collapses. Emulsion stability is a measure of the stability of the emulsion in presence of stress factors such as heat, blending, and centrifugation (16,17).

Foaming properties are important for the use of proteins in cakes, whipped toppings, and frozen desserts. In this case the parameters that are taken into account are foam volume and foam stability (measured as the amount of foam that remains after a definite period of time), usually as a function of pH and temperature (16,17).

Juiciness, texture, binding of structure, and appearance are some of the qualities of food items that depend on the fat and water absorption properties associated with proteins. Interactions between proteins and water are important to such characteristics as hydration, swelling, viscosity, and gelation; entrapment of fats in meat emulsions, flavor absorption, and dough preparation are a few of the aspects related to the interactions between proteins and lipids. Fat absorption and water absorption are generally evaluated by measuring the amount of oil or water retained by the proteins under defined conditions.

Effects of SFE on Proteins

Pressure alone or extraction with SC-CO_2 may cause denaturation of protein, destruction of individual amino acids, or loss of enzymatic activity. Proteins, peptides, and amino acids are polar materials showing little if any solubility in SC-CO_2 and after extraction they are found in the residuals (18). Ideally, if the residue is to be consumed as a food, no alteration should occur during extraction with the supercritical fluid, or at least functional and nutritional properties should be preserved. Unfortunately, many factors influence the effect of supercritical fluids on proteins and enzymes, including moisture, temperature, pressure, and residence time in the extractor as well as the material to be extracted or treated itself.

In two different studies Weder reported that, in the presence of water, proteins treated with carbon dioxide or nitrogen at 30 MPa and 80°C undergo unfolding, partial oligomerization, and some fragmentation (19–20). However, these alterations are not related to the kind of gas utilized but to the concurrent presence of heat and water.

Weder studied also the influence of SC-CO_2 on some amino acids with isoelectric points ranging from 3.2 to 9.7 (19). The experimental data show that these components do not react with carbon dioxide at supercritical conditions. Of the amino acids tested, only L-glutamine showed some alteration, being partially converted to 2-pyrrolidinone-5-carboxylic acid. However this conversion can occur even in the absence of carbon dioxide; it is caused by heat alone. On the basis of these results, Weder concluded that deterioration to proteins and amino acids should not occur under the conditions likely to be employed in SFE of foods.

Results of other studies conducted on some pure proteins (cholesterol oxidase, α-chymotrypsin, lysozyme, penicillin amidase, ribonuclease, and trypsin) treated with carbon dioxide at mild temperatures and pressures (35 to 80°C, 10 to 30 MPa) suggest that under static conditions (2 to 24 h) these substances suffer minimal damage as long as water is absent from the system (18). Cholesterol oxidase showed little or no denaturation at 35°C and 10 MPa, as measured by electron paramagnetic resonance spectroscopy. After treatment with humid SC-CO_2 at 37°C and 10 MPa, fluorescence emission spectroscopy showed a partial unfolding of α-chymotrypsin, penicillin amidase, and trypsin, although gel electrophoresis failed to show any oligomerization or fragmentation. Treatment of lysozyme and ribonuclease with humid SC-CO_2 at 80°C and 30 MPa caused some denaturation of these proteins, as evidenced by increasing tryptic digestibility; furthermore, some oligomerization and fragmentation were detected by gel electrophoresis. However, available lysine content and amino acid composition were unaltered.

Soybean proteins

Eldridge et al. (21) extensively studied the extraction of soy flakes with SC-CO_2 under conditions where triglycerides show complete miscibility in the supercritical fluid. The effects of SC-CO_2 extraction conditions on nitrogen solubility index (NSI), flavor score, and enzyme activity of soy flakes in this study are shown in Table 13.1. Where

high NSI and flavor scores are desired, 81.6 MPa and 85°C give the best results at moisture levels of 10.5 to 11.5%. NSI and flavor scores approached 70 and 7 respectively. Lipoxygenase activity was usually destroyed at moisture levels above about 6.5%, but urease activity remained unaffected in all 20 samples, since a consistent pH change of 2.0 to 2.1 was observed throughout. The usual grassy-beany and bitter flavors of hexane-defatted soybean flakes were only minimally detectable in the optimally SC-CO_2 extracted materials. Bland defatted soybean meal prepared by SC-CO_2 extraction was further processed into high-quality protein concentrates and isolates that were shown to be stable when stored under adverse conditions.

TABLE 13.1 Effect of SC-CO_2 Extraction Conditions on NSI, Flavor Score, and Enzyme Activity of Soy Flakes (21)

	Extraction conditions[a]						
Sample	Pressure (MPa)	Temperature (°C)	H_2O (%)	NSI[b]	FS[c]	LU[d]	TI[e]
1	78.2	100	9.0	48	6.9	0	17.5
2	78.2	90	5.0	82	4.8	589	31.3
3	78.2	90	9.0	67	6.3	3	21.6
4	78.2	90	9.0	68	6.6	0	19.4
5	78.2	90	9.0	57	6.7	8	19.9
6	78.2	90	9.0	65	7.2	0	27.3
7	78.2	90	9.0	69	6.2	0	27.5
8	78.2	90	9.0	69	6.0	ND	27.7
9	78.2	90	12.4	62	6.4	16	29.5
10	78.2	80	9.0	80	5.8	779	31.3
11	74.8	84	6.5	80	4.2	688	ND
12	74.8	84	11.4	63	6.1	57	27.3
13	74.8	96	6.5	67	6.8	0	22.8
14	74.8	96	11.4	33	7.2	0	10.0
15	81.6	84	6.5	81	5.6	625	21.4
16	81.6	84	11.4	62	7.2	3	24.8
17	81.6	96	6.5	72	6.0	0	28.2
18	81.6	96	11.4	34	7.3	0	ND
19	72.1	90	9.0	66	6.3	10	27.7
20	84.4	90	9.0	69	6.6	80	27.4

[a]In all extractions, time and CO_2 flow remained constant.
[b]Nitrogen solubility index (NSI), a measure of protein solubility.
[c]Flavor score: 1 to 10 where 1 is strong and 10 is bland.
[d]Lipoxygenase units: μM O_2 consumed/min/mg protein.
[e]Soybean trypsin inhibitor activity, mg/g.
ND = not determined.

Various investigators have studied the effects of SC-CO_2 extraction conditions on the activity of the trypsin inhibitor present in soybeans (21–24) the results are summarized by Weder (18). At moisture levels of 9.8% and extraction conditions of 40 to 50°C and 13.5 to 68.9 MPa, no trypsin inhibitor inactivation was detected after extended reaction times. Pancreatic hypertrophy, changes in digestive enzymes, and reduced growth rate were observed in chicks fed the extracted meals. However, by careful control of the moisture content of the flakes, temperature, and pressure during extraction, the trypsin inhibitors could be completely inactivated (Table 13.1). Judicious control of moisture, temperature, and pressures during SFE of soy flakes could also inactivate lipoxygenase, which can catalyze the formation of lipid hydroperoxides known to be precursors of undesirable flavors. However, SFE conditions that destroy both trypsin inhibitors and lipoxygenase resulted in considerable protein denaturation (Table 13.1, run 18), as shown by the low NSI value.

Treatment with SC-CO_2 does not prove efficacious in some instances. Sessa et al. (24) contrasted SC-CO_2 with hexane defatting of whole and cracked soybeans and found no apparent differences in trypsin inhibitor activity after steeping the beans in 0.1 M Na_2SO_4. The SC-CO_2 extraction conditions of 85 MPa and 84°C for 15 min were not sufficient to deactivate the enzyme. These investigators also claimed that SC-CO_2 treatment did not reduce the residual sulfite levels in the steeped beans.

Corn Germ Proteins

Dry milled corn germ (CG) was extracted with SC-CO_2 at 50°C and pressures ranging from 34 to 54.4 MPa (25). Flavor evaluation of the resulting meals, against hexane-extracted controls showed that SC-CO_2–extracted meals were superior; after tempering to 8% moisture followed by an additional extraction, further improvement in flavor was achieved. Two factors are responsible for these observations.

1. SC-CO_2 is much more effective in removing triglycerides and bound lipids than hexane is.
2. Residual triglycerides left in extracted meals can be oxidized either by air or enzymes, giving rise to grassy, beany, bitter flavors.

The bound lipid content of hexane-extracted meal was nearly four times that of the SC-CO_2–extracted flour. The improvement in flavor afforded by SC-CO_2 extraction can be attributed to peroxidase inactivation as well as to the lowered lipid content. Peroxidase activity is normally difficult to destroy by the normal toasting process; however, Christianson et al. (25) reported that treatment under supercritical fluid extraction conditions resulted in a sevenfold reduction in peroxidase activity. Some protein denaturation was observed, as shown by reduction in nitrogen solubility index of the SC-CO_2–extracted flours. The amino acid profile of protein in both SC-CO_2–extracted and hexane-extracted CG flours had an excellent balance of essential amino acids and compared favorably with published FAO/ALTO standards for highly nutritious proteins.

SFE-defatted corn germ proteins can exhibit quite different fat-binding capacity and water retention properties, from the proteins prepared by hexane defatting. Lin and Zayas (26) found the SFE germ to be whiter—less red and less yellow—than corresponding germ defatted with hexane. Although temperature-dependent, the fat-binding capacity and water retention of the SC-CO_2–produced CG was significantly better than those of the solvent-extracted germ. Fat-binding capacity could be made equivalent for both germs by the application of heat. Both germs exhibited higher protein solubility at higher pH. Some of these observed differences may be attributed to the lower lipid level in the starting SC-CO_2–treated germ (almost 10% lower than in hexane-processed germ) or to morphological differences in germ structure.

Tömösközi et al prepared defatted CG proteins using hexane and SC-CO_2 (26 MPa/60°C); to better characterize the influence of the SFE treatment, these authors also studied the properties of alkaline-extracted protein isolates prepared from the defatted CG meals. Compared to meal prepared with hexane extraction, SC-CO_2–defatted meal showed a slightly higher *in vitro* nutritional value, but this difference disappeared in the isolates. The emulsion activity of meals and isolates was similar for both extraction procedures, but the emulsion stability of the meals was very poor compared to that of the isolates. The supercritical extraction process did not significantly affect the solubility profile of the treated proteins, but it negatively influenced their foaming properties. In contrast with the results obtained by Lin and Zayas (26), water and oil absorption values did not differ significantly between CG proteins defatted with SC-CO_2 and with hexane.

Other similar corn-based substrates treated with SC-CO_2 also exhibited improved properties critical to their incorporation into food systems. Wu et al. (28) evaluated corn distillers' dried grains (CDG) after extraction with SC-CO_2 and showed that the extracted CDG had lower fat and higher neutral detergent fiber content relative to untreated CDG. Perhaps more important were the acceptable flavor scores given the CDG substrate after SC-CO_2 treatment, as summarized in Table 13.2. Although individual flavor descriptions (cereal/grain, fermented, astringent) were higher than those of a standard substrate (untreated wheat flour) and in some cases those of untreated CDG, the overall flavor scores for the SC-CO_2 CDGs were significantly blander than those for untreated CDG. These results were achieved by using only small amounts of GRAS (Generally Recognized as Safe) cosolvents (water or ethanol) with the SC-CO_2.

Wu et al. (29) similarly characterized corn gluten meal that had been extracted with SC-CO_2 versus hexane/ethanol-treated meal. Extraction pressures ranged from 13 to 68 MPa, while extraction temperature was varied from 40 to 80°C. The resultant properties of the extracted meals were found to depend somewhat on the meal particle size, which influenced the degree of lipid removal, particularly since oil levels are extremely low in gluten meal (approx. 2%). Both SC-CO_2 treatment and hexane/ethanol-extraction of the meal were found to decrease the intensity of the fermented off-flavor significantly.

TABLE 13.2 Flavor Scores and Descriptions for Corn Distillers' Grains (CDG) Defatted with Supercritical Carbon Dioxide at 81.6 MPa (28)

Treatment	Flavor score[a]	Flavor description[b]		
		Cereal grain	Fermented	Astringent
Wheat flour				
Untreated	8.0 D	0.8	0	0
CDG				
Untreated	5.0 G	0.5	2.0	0.6
86°C, 1 mL ethanol	5.9 F	0.7	1.4	0.4
86°C, 1 mL H_2O	6.7 E	0.8	1.0	0.5
86°C, 1 mL ethanol, 1 mL H_2O	6.4 E	0.8	1.2	0.3
101°C, 1 mL ethanol	5.8 F	0.7	1.3	0.6
101°C, 1 mL H_2O	6.3 E	0.7	1.2	0.2
101°C, 1 mL ethanol, 1 mL H_2O	6.4 E	0.7	1.2	0.4

[a]Based on a 1–10 scale with 10 = bland, 1 = strong flavor. Flavor scores with no common letter (D–G) are significantly different (95% confidence level, $P < 0.05$). Least significant difference = 0.6.

Sunflower Proteins

In recent years several papers have been published on the extraction of sunflower oil with SC-CO_2 but only a few have reported the effects of the extraction process on the residual protein (27, 30–34). Studies conducted over a wide range of pressures (20 to 70 MPa) and temperatures (40 to 80°C) showed that the highest solubility of sunflower oil in the supercritical fluid was reached at 80°C and 70 MPa, similar to the SFE of other seed oils (31). At these conditions it was possible to remove more than 90% of the available oil contained in 280 g of seeds in only 6 min.

In subsequent research, Favati et al. (34) studied the effects on the proteinaceous substrate, conducting the SFE process at two sets of pressure and temperature conditions (20 MPa/40°C vs. 70 MPa/80°C) chosen to have a low and high impact on the matrix. Hexane extraction was also performed on the sunflower seeds for comparison purposes. Compared to the raw sample, both SFE and hexane extraction procedures reduced the sunflower protein solubility over the pH range 1.5 to 8.0, while only the sample extracted at 20 MPa and 40°C showed a slightly higher solubility at higher pH values (Fig. 13.1*a*). For protein solubility as a function of the ionic strength of the solution in which the sunflower proteins were solubilized, the hexane-treated meal and the unextracted sample showed similar profiles, whereas the meals extracted with SC-CO_2 had slightly higher solubilities above μ = L (Fig. 13.1*b*). Water absorption

data were similar for both the meals treated with hexane and those treated with SC-CO_2, while fat absorption levels were somewhat inversely related to the amount of lipids left in the matrix after the extraction. The meals treated at 20 MPa and 40°C had a residual oil content of about 32% and a fat absorption level lower than that of samples extracted with hexane or with SC-CO_2 at 70 MPa and 80°C.

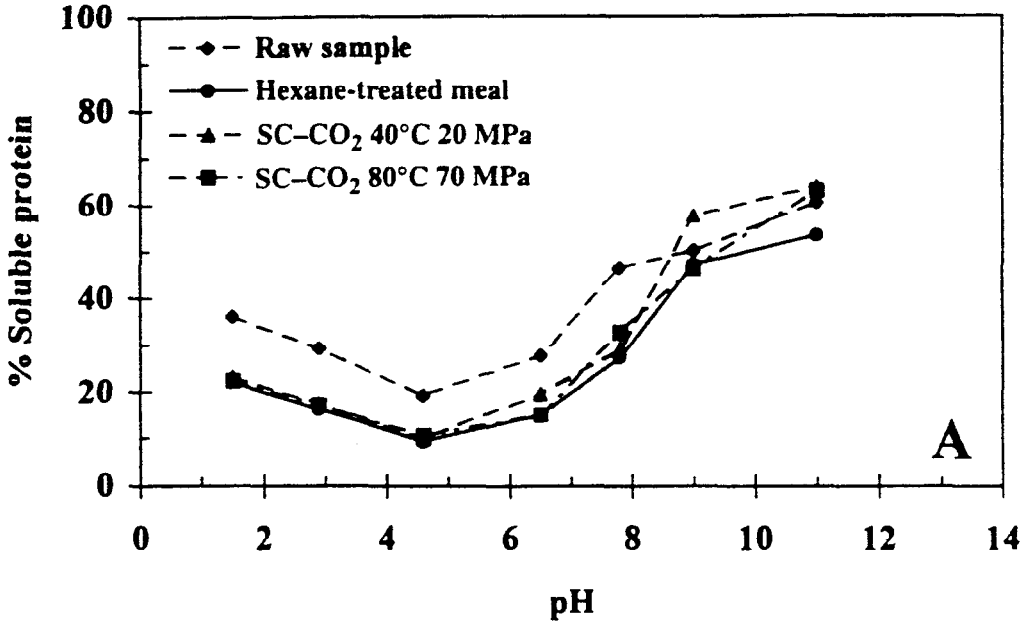
Similar results were reported by Tömösközi et al. (27) for sunflower proteins extracted with CO_2 at 30 MPa and 40°C. The protein solubility was not affected by the SFE treatment; fat absorption was lower in comparison to the hexane-extracted counterparts, because of partial removal of the native oil. The emulsion activity and emulsion stability of the SC-CO_2 extracted meals were slightly higher, but foam activity and foam stability were adversely affected by the supercritical extraction.

Peanuts and Peanut Butter

Santerre et al. (35) extracted roasted peanuts with SC-CO_2 at 41.36 MPa and temperatures of 50 to 60°C for up to 4 h. The extracted peanuts were evaluated for shatter, color, shear compression force, moisture, lipid content, and sensory attributes. The extracted peanuts were also processed into peanut butter and evaluated for color, relative torque resistance force ratio, and sensory attributes. After 4 h extraction SFE reduced the lipid content from 51.6 to 40.6% and the moisture from 1.4 to 1.2%. Peanut shatter was highest after 4 h extraction, but no trend was observed between shatter and extraction time. The color of peanuts was affected by SFE extraction time. This apparent bleaching effect was observed on the outside of partially extracted peanuts and was associated with lipid removal from the kernels. The color of the peanut butter was consequently affected by SFE but these changes were small. The shear compression force of peanuts increased with extraction time; other workers had demonstrated a corresponding inverse correlation with sensory crispness (36). The peanut butter also showed increased relative torque resistance with extraction time, attributed to reduction of oil content in the peanut butter. Sensory evaluations made on SFE-extracted peanuts showed a reduction in roasted-peanut aroma, fracturability, and moistness with increasing extraction time. Similar evaluations made on peanut butter showed increased adhesiveness but no trends in aroma intensity with extraction time. The authors concluded that because SC-CO_2 does not extract phospholipids, SFE of oil from roasted peanuts could potentially result in extended storage stability of the residual peanuts.

Miseellaneous vegetable proteins

Stahl et al. (37) extracted soy flakes, lupine seed, cottonseed, and jojoba meals with SC-CO_2 at 40°C and 35 MPa. Control extractions were made with hexane. The proteins were isolated and characterized by gel electrophoresis. The results showed no significant difference in the relative proportions of the subunits or reduced polypeptides recovered from CO_2-treated meals compared to the respective hexane-extracted control. In addition, there was no evidence of protein crosslinking. Amino acid analysis also showed no significant differences between the control and the CO_2-extracted samples.



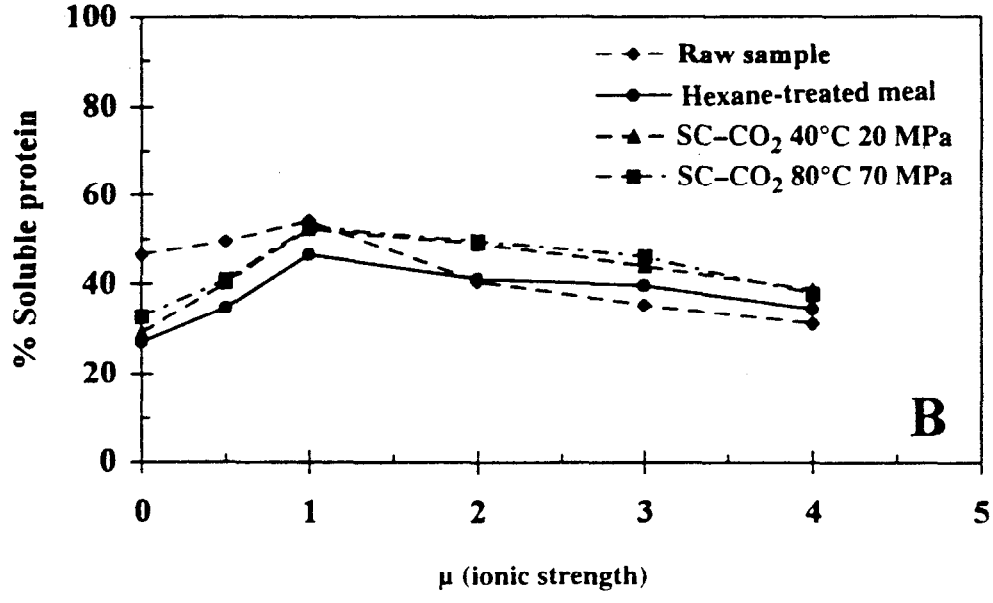


Fig. 13.1. Protein solubility profile of sunflower meals extracted with hexane and SC-CO_2 as a function of pH (*a*) and ionic strength (*b*). (Source: Favati, F., et al., in *Fluid: Supercritici e le laro Applicazioni,* edited by I. Kikic and P. Alessi, Centro Stampa dell' Università degli Studi di Trieste, Trieste, Italy, 1995, pp. 121–128.)

Tömösközi et al. (27) prepared protein isolates from SC-CO_2–extracted wheat germ and pumpkin seed meals and compared them to hexane-extracted counterparts. The *in vitro* nutritional values of the SC-CO_2– and hexane-treated samples were similar, while the content of cysteine and lysine in the whole-protein meals was higher in the SFE materials; however, after conversion to isolate this difference decreased. The two different oil extraction procedures did not cause any appreciable effect on the protein solubility profiles of meals and isolates; only pumpkin seed isolates treated with the supercritical fluid showed a better profile. Analytical data for emulsifying activity, emulsifying stability, water absorption, and fat absorption did not allow definite conclusions to be drawn. Foam activity of all the samples was low but acceptable, but foam stability was adversely affected by the extraction with SC-CO_2.

Special Applications

Previous sections of this review have been primarily concerned with the characterization and use of proteins and matrices that represent commodity agricultural products treated with SC-CO_2. This section focuses on a variety of substrates that illustrate the potential of SFE for producing a desired effect on the product remaining after the extraction. The coverage is probably not exhaustive but will allow the reader an appreciation of how widespread the application of supercritical fluids as "treatment" agents in processing foodstuffs can be. Of course, many of the cited studies are at best bench-scale in scope, and no claims can be made for the practicality or economic viability of their application.

Protein Hydrolyzates

Froschl et al. (38) have reported on lipid removal from protein hydrolyzates using SC-CO_2 as well as SC-CO_2/ethanol mixtures in a pilot plant whose upper pressure limit was 32.5 MPa. Both a protein hydrolyzate of high viscosity and a powder were extracted; however, a specific description of the matrices was lacking because of their proprietary nature. The target value was 97% removal with minimal loss in nitrogen content; the traditional process, using methylene chloride as the extraction solvent, resulted in a loss of 20% of the nitrogen content.

Despite attempts to increase the fat solubility in the SC-CO_2 by increasing both the temperature and pressure (to 60°C and 30 MPa, respectively), the targeted removal value could not be obtained. In addition, foaming problems occurred in the extractor; to eliminate foaming a demister was installed in the extraction vessel. Introduction of approximately 6 wt % ethanol into the SC-CO_2, however, resulted in over 90% of the lipids being removed; a 96.3% decrease was achieved at 30 MPa and 60°C. However, this gain was offset by a reduction in nitrogen value, even when using SC-CO_2 alone. High levels of amino acids were found in the ethanol phase of the extract after SFE, a result in stark contrast with earlier reported studies. Subsequent experiments with ethanol-laden SC-CO_2 showed that amino acids were not solubilized to any appreciable extent in this mixture; hence, the investigators theorized that the amino acids reached

the collection vessel via entrainment with lipoproteins or water present in the sample. This nonspecificity in the extraction forced the investigators to abandon the SFE process, but their results suggest that a SC-CO_2 approach may have applicability in dissolving low-molecular-weight proteins or peptides from natural products. Such dissolutions have been demonstrated, particularly in supercritical fluid chromatography (39) or using ultrahigh-pressure SC-CO_2 above 70 MPa (40).

Meat

Extraction of fat from meat has been covered in Chapter 11. Many of these studies have characterized both extract and residual substrate in terms of fat or moisture content, but few note the effect of SFE on the proximate values, such as protein and ash. King et al. (41) extracted preformed beef patties with SC-CO_2 varying the extraction conditions, such as the moisture level of the meat patty, pressure, temperature, precooking, and spatial orientation of the patties in the extractor vessel. The results are reported in Table 13.3 on a dry matter basis for both control and extracted beef patties.

Higher extraction pressures tended to increase the protein content of the patties as fat and moisture content was reduced. Ash content, on the other hand, was not appreciably affected. Contrast analysis of the results given in Table 13.3 yields the trends noted in Table 13.4. Precooking, for example, increased the protein and ash content, as might be expected, while decreasing the cholesterol of the patties. The freeze-drying process, crucial to the removal of fat in meat products with SC-CO_2 (42), had no effect on fat, protein, or ash content.

Contrast analysis of control versus extracted patties suggested that protein and ash were significant altered at a P value of less than 0.05. The contrast of extraction pressure was significant (17.0 *vs.* 54.4 MPa) for sample fat, protein, and ash content. This suggests that a patty high in protein may be produced by SFE with CO_2.

Eggs

Dried eggs find a wide use in the food industry, and of the utmost importance is the retention of their functional properties, which are mainly related to the characteristics of the native proteins and to the presence of phospholipids. In the last few years treatment of dried egg yolk with SC-CO_2 for removal of cholesterol and lipids has been studied by several authors (43–46) and recently a low-cholesterol egg formulation obtained by using SC-CO_2 has become available on the market.

Froning et al. (43) utilized pure CO_2 to extract cholesterol and lipids from dried egg yolk at four different levels of pressure and temperature (16.3 MPa/40°C; 23.8 MPa/45°C; 30.6 MPa/45°C; 37.4 MPa/55°C). The effects of the extraction process on the substrate were assessed, particularly some functional properties of the matrix; emulsion stability (mayonnaise test) and sponge cake volume.

The proximate composition of the treated samples showed that the extraction of the lipids was influenced by the operating conditions; at 37.4 MPa and 55°C more than one-third of the lipid fraction could be removed. Protein content increased

TABLE 13.3 Protein, Ash, Fat, and Cholesterol Content of Control and Supercritical CO_2–Extracted Beef Patties (41)

Treatment[a]	Extraction pressure (MPa)	Protein (%)	Ash (%)	Fat (%)	Cholesterol (mg/100 g)
Raw control		49.19	2.38	48.42	177.09
Raw static	17.0	56.10	2.11	41.79	193.80
Raw dynamic	17.0	55.24	2.24	42.52	196.56
Raw dynamic	54.4	56.82	2.30	40.88	194.38
Raw FD[b] control		44.78	2.03	53.19	167.48
Raw FD static	170	48.00	2.22	49.79	154.07
Raw FD dynamic	17.0	52.19	2.35	45.45	145.79
Raw FD dynamic	54.4	70.49	3.16	26.34	102.97
Cooked control		59.78	1.98	38.25	189.33
Cooked static	17.0	71.20	2.56	26.213	186.55
Cooked dynamic	17.0	72.89	2.62	24.49	182.95
Cooked dynamic	54.4	77.17	2.77	20.06	176.24
Cooked FD control		62.59	2.20	35.22	194.34
Cooked FD static	17.0	62.91	2.33	34.76	163.12
Cooked FD dynamic	17.0	69.13	2.58	28.30	134.98
Cooked FD dynamic	54.4	83.00	3.15	13.85	100.54

[a]Extraction method: "static" = CO_2 is not replenished; "dynamic" = CO_2 is replenished.

because of the decrease in lipid concentration; the moisture level changed with no logical correlation with the extraction parameters. The cholesterol content of the dried egg yolk was reduced by at least 16% when the extraction was conducted in the mildest conditions, and approximately 66% of the total cholesterol was removed when operating at 30.6 MPa/45°C and 37.4 MPa/55°C. The extraction with SC-CO_2 apparently did not cause any reduction in the total phospholipid content—a desirable effect, considering the importance of these components for the emulsifying properties of eggs. The emulsion stability of mayonnaise prepared with the eggs treated with SC-CO_2 did not show significant differences ($P<0.05$) for the different treatments when compared to a control sample. Only for the eggs extracted at 37.4 MPa/55°C was a definite ($P<0.05$) reduction measured in the emulsion stability. Supercritical fluid extraction significantly ($P<0.05$) improved the sponge cake volume values, except for the extractions conducted at 37.4 MPa and 55°C. The authors attributed the decrease in sponge cake volume to partial denaturation of the egg proteins under the extraction conditions. However, electrophoretic analysis of the samples did not show any appreciable protein fraction modification that could be ascribed to the extraction conditions.

Rossi et al. (44) studied the extraction of lipids and cholesterol from egg yolk powder at 15 MPa and 50°C with pure SC-CO_2 and with the addition of ethanol at 1, 3, and 7% (w/w) level. About 50% of the available cholesterol was removed when the

TABLE 13.4 Contrasts of Extraction Parameters for SC-CO_2–Extracted Beef Patties (41)

Variable	Contrast comparisons			
	Raw *vs.* cooked	FDa *vs.* NFD[b]	Control *vs.* extracted	17 MPa *vs.* 54.4 MPa
Dry matter basis:				
Δ Fat[c]	0.13	0.86	•••	•••
Δ Cholesterol[d]	•••[h]	•••	0.29	0.12
Cholesterol	0.46	•••	0.28	0.11
Fat	•••	0.43	••[g]	•••
Protein	•••	0.35	0.08	•••
Ash	•••	0.09	••	•••
Moisture-retained basis				
Δ Fat[e]	0.12	•••	••	•••
Δ Cholesterol[f]	•••	•••	0.23	0.09

[a]FD = Freeze-dried.
[b]NFD = not freeze-dried.
[c]Difference in percentage fat from control on a dry matter basis.
[d]Difference in percentage cholesterol from control on a dry matter basis.
[e]Difference in percentage fat from control on a moisture-retained basis.
[f]Difference in percentage cholesterol from control on a moisture-retained basis.
[g]•• = significant difference ($P < 0.05$)
[h]••• = significant difference ($P < 0.01$).

ethanol concentration reached 7%. However, the presence of ethanol lowered the selectivity between cholesterol and some phospholipids, thus causing extraction of lecithin. Furthermore, the addition of ethanol led to a slight extraction of proteins.

The presence of an alcohol as entrainer can also denaturate of the proteins contained in the egg yolk, as demonstrated by Arntfield et al. (45), who extracted freeze-dried egg yolk with SC-CO_2 at 36 MPa and temperatures of 40, 55, 65, and 75°C and used differential scanning calorimetry to evaluate protein conformation changes that could occur as a result of the SFE process. The observed denaturation indicated that over the range of temperatures studied, SFE with pure CO_2 did not affect the thermal stability of the proteins. However, the enthalpy of denaturation (ΔH) for ovalbumin was significantly lower for the extraction conducted at 75°C. This effect on the protein conformational changes was then shown to result from the combination of pressure and temperature: Heating the egg yolk at 75°C in presence of air or CO_2, but not under pressure, did not cause any change in the ΔH value.

The same authors then studied the stability of the egg yolk proteins in the presence of an entrainer by adding methanol to the CO_2. These experiments were conducted at 40°C and 36 MPa with a 3% methanol concentration in CO_2. Differential scanning calorimetry analysis revealed that the presence of methanol caused significant conformational changes in the egg protein. The resulting ΔH value for ovalbumin was 50% lower than that of a similar sample treated with pure CO_2.

Rice

Defatting has also been practiced on several other substrates to enhance their end use. Taniguchi et al. (47) extracted rice and rice koji (used for brewing applications) with SC-CO_2 at 30 MPa and 40°C so as to produce a substrate similar in lipid content to polished rice. As in the case of the protein hydrolyzate, addition of ethanol removed more lipid. The removal of lipids from rice koji did not affect the overall enzyme activity of the rice, but acid carboxypeptidase in particular was found sensitive to the SC-CO_2/cosolvent treatment. However, unlike the treatment of rice with neat CO_2, the SFE processing of rice koji did not improve the quality of the resultant sake. (See Chapter 14.)

Mustard seeds

Oils can contribute to the deterioration of spices such as mustard seed by oxidation. Any defatting of mustard seeds must be accomplished without lowering the activity of myrosinase, an enzyme essential to the generation of the acrid component of mustard (allylisothiocyanate). As with the case for rice, SFE with CO_2 at 30 MPa and 40°C (48) permitted up to 90% of the oil to be extracted, without decreasing myrosinase activity or the concentration of its substrate (synigrin). Pretreatment of the mustard seeds was found to be critical for achieving a successful deoiling; fully pressed seeds yielded the optimum result. (See Chapter 14.)

Leaf Protein Concentrates

Removal of lipids and lipophilic components by SFE has been studied by Favati et al. (49,50) for the production of alfalfa leaf protein concentrates (LPC) for human consumption. LPC have a high protein content and good amino acid composition, but there are major obstacles to their inclusion in the human diet, particularly their color and strong grassy flavor. The former is due to the presence of chlorophylls and carotenoids, the latter to the lipid fraction and to the products of oxidation of the unsaturated fatty acids.

The same authors (50) studied the effects of SFE treatment on some functional properties and organoleptic characteristics of the LPC in comparison with those of LPC treated with acetone. The extractions were conducted at four different pressure levels (10, 30, 50, and 70 MPa) and at the temperature of 40°C. Acetone extraction proved to be more effective for lipid removal; the maximum extraction of fat with SC-CO_2 was attained at the pressure of 70 MPa (Table 13.5). Lipid extraction resulted in correspondingly increased protein content, whereas the ash level was only slightly affected by the SFE process. Water absorption data were not significantly different for the untreated LPC and the samples extracted with the two solvents. Conversely, the treatment with SC-CO_2 caused a definite reduction in the amount of oil absorbed. The residual meals were further characterized in reference to the solubility of the proteins over the pH range 3 to 11. In comparison with the untreated LPC, acetone extraction

significantly ($P < 0.05$) reduced the protein solubility, whereas SFE did not appreciably affect this functional property. Sensory evaluation of the treated samples indicated that the grassy flavor was definitely reduced in both SC-CO_2– and acetone-extracted meals. Despite the almost complete removal of the carotenoid pigments (Table 13.5), SC-CO_2–treated LPC did not show a significant reduction in the color, which was evaluated as "deep green," whereas acetone extraction resulted in a "white-gray" residue. This difference can be attributed to the presence in the LPC of chlorophylls and pheophytins, which at the tested SFE conditions exhibited only minimal solubility in the supercritical fluid (49).

Fish

SFE has also been investigated for the removal of lipids from the muscle of several species of fish, with the main goal of obtaining a proteinaceous residue having chemical, physical, and organoleptic characteristics suitable for its use for human consumption (51–53).

Hardardottir and Kinsella have reported on the extraction of lipids and cholesterol from rainbow trout (*Salmo gairdneri*) muscle using neat and ethanol-enriched (10% by weight) SC-CO_2 (51). The experiments were conducted at 40 and 50°C over the pressure range 13.8 to 34.5 MPa. The addition of ethanol to the stream of CO_2 did not cause a significant increase in the removal of cholesterol; conversely, in the presence

TABLE 13.5 Composition and Properties of Untreated LPC and Residual LPC after Extraction with Acetone and SC-CO_2 (49, 50)

		Extracted LPC[a]				
			Extraction pressure (MPa)			
	Untreated LPC	Acetone	10	30	50	70
Proximate composition (dry basis):						
Crude protein (N × 6.25) (%)	46.2	56.1	48.0	50.6	51.1	51.2
Crude fate (%)	13.0	0.2	7.5	6.5	6.2	5.4
Ash (%)	12.8	15.6	13.2	14.1	14.2	13.9
Functional properties:						
Water absorption (g H_2O/g LPC)	2.90	3.04	2.95	2.93	2.95	2.82
Fat absorption (g oil/g LPC)	2.33	2.31	1.81	1.84	1.89	1.87
Carotenoid recovery:						
Carotene extracted (%)	–	–	9.19	95.98	96.47	98.48
Lutein extracted (%)	–	–	0.52	29.52	61.23	70.20

[a]Extraction temperature 40°C.

of the entrainer the amount of lipids recovered rose from 78 to 97%, with extraction phospholipids as well. SFE caused some denaturation of the proteins, as indicated by the reduction of the solubility of muscle protein; electrophoretic analysis revealed that the extraction process affected myosin especially. As a consequence, the residual meals showed poor emulsifying properties and did not form gels when heated.

Fujimoto et al. (52) attempted to use liquid and supercritical CO_2 to defat sardine (*Sardinops melanosticta*) meat powder as an alternative source of surimi (minced meat) which is utilized in Japan for the production of Kamaboko. (The problems related to the use of sardines for surimi include the high oil content, the strong characteristic odor, and the low thermal stability of the myofibrillar proteins of this fish.) The extractions were conducted at 25 MPa and at temperatures of 12 and 40°C. Both treatments successfully reducing the lipid content, but the quality of Kamaboko produced with sardine defatted with SC-CO_2 was absolutely inferior. This result was ascribed to the negative influence of the higher extraction temperature on the proteins. The authors then investigated the effect of the addition of a modifier (10% ethyl acetate) to the liquid CO_2. The degree of denaturation of the proteins was evaluated by measuring the soluble nitrogen and the ATPase activity of the defatted minced sardine meat. Extraction with neat liquid CO_2 reduced the nitrogen solubility value, and a further decrease was caused by the use of the modifier. ATPase activity was even more significantly affected by the presence of ethyl acetate. The sardine meat powder defatted with liquid CO_2 allowed the production of Kamaboko of good quality, having a faint sardine odor and good storage stability, while the addition of the organic solvent resulted in a Kamaboko of much lower quality and having a dry and heated sardine odor.

Temelli et al. (53) applied SC-CO_2 to remove the oil contained in the muscle of Atlantic mackerel (*Scomber scombrus*) (53). In this research the use of SC-CO_2 had two objectives: recovery of a lipid fraction rich in ω-3 fatty acids, and production of a proteinaceous residue having potential uses for food applications. The experiments were conducted at three different levels of pressure (20.7, 27.6, and 34.5 MPa) and temperature (35, 45, and 55°C), and as a reference the fish muscle was also defatted with hexane. The effects of the extraction conditions on the water-binding potential (WBP) of the residual proteins were then evaluated. Both SFE and hexane extraction caused an increase of the WBP values; however, the WBP of the SC-CO_2–extracted proteins was always lower than that of hexane-extracted proteins. WBP appeared to be related to the amount of lipids removed from the substrate and to changes in protein functionality. The authors attributed this behavior to an increase in the available binding surface area of the protein due to the lipid removal, as well as to changes in the protein conformation.

Phospholipids Concentration and Isolation

Often treatment of a substrate with SC-CO_2 is designed to remove or enrich a specific component, such as phospholipids in eggs or oilseeds. Bulley (Chapter 9) has covered the application of SFE to eggs, primarily to remove cholesterol from the substrate and

to increase its protein and phospholipid content. Here we shall focus on attempts to remove phospholipids from matrices after initial defatting so as to recover a value-added product that would otherwise be wasted.

In a previous chapter it was noted that a highly viscous medium such as lecithin can be deoiled by applying a special technique known as "jet extraction" (54). Here the surface area–to-volume ratio of the substrate to be deoiled is maximized by extrusion through a narrow orifice. The droplets or "strings" of lecithin are then immediately contacted by SC-CO_2, which can effectively deoil the lecithin if the contact time is sufficient. A deoiled powder can result by using this approach if all parameters are optimized. Eggers and Wagner (55) have recently studied the original process (54) in detail and offer an alternative method to achieve the same result.

Perhaps the first to specifically address the removal of phospholipids after oil extraction from seed matrices was Temelli (56). She demonstrated what previous investigators had shown, that addition of a cosolvent (entrainer) such as ethanol to SC-CO_2 enhanced the total recovery of lipid matter on a mass base while increasing the phospholipid content of the extract. Both canola flakes and press cake were extracted typically at 62 MPa and 70°C using ethanol mixed with the canola substrates at 5 to 15 wt.% addition. Ethanol was also added to the canola substrates after initial deoiling of the flakes with SC-CO_2, since equipment for continuous addition of ethanol to the SC-CO_2 was not available in this study. This principle has also been practiced in the decaffeination of coffee, where water is added to the beans to enhance extraction of caffeine specifically from the bean matrix.

Dunford and Temelli (57) expanded on these original studies to better characterize the phospholipid recovery from deoiled canola. Again, enhanced recovery was experienced by soaking the canola meal in ethanol after initial deoiling of the canola substrate. A phospholipid recovery level of only 21% was reported for extraction at 55 MPa and 70°C using SC-CO_2 with 8 mol%, provided continuously by a second cosolvent pump. Presoaking of the canola meal prior to SC-CO_2/ethanol extraction yielded 30% recovery of available phospholipids, according to these researchers. SC-CO_2/ ethanol extraction of an acetone-insoluble (AI) fraction yielded a 50% phospholipid-containing extract utilizing ethanol presoaking of the AI fraction. In all of the above cases, phosphatidylcholine (PC) was the major phospholipid recovered. Sample mass balances with accompanying phospholipid yields are presented in Fig. 13.2. This flow schematic could serve as the basis of a future process, designed to accommodate GRAS-approved processing agents (CO_2, ethanol) in several of the described stages. A similar approach has been described by Manohar et al. (58) using response surface methodology to optimize the fractionation of SC-CO_2–deoiled soybean lecithin, except that these researchers used ethanol rather than SC-CO_2/ethanol for PC enrichment.

More recently, Montanari et al. (59) have reported on the recovery of the phospholipid fraction left over from the exhaustive deoiling of soybean flakes. Their approach has been to deoil soybean flakes at 70 MPa and 80°C, which results in nearly complete removal of the soybean oil and retention of the phospholipids by the soybean meal. These investigators then utilized SC-CO_2 with ethanol as a cosolvent to

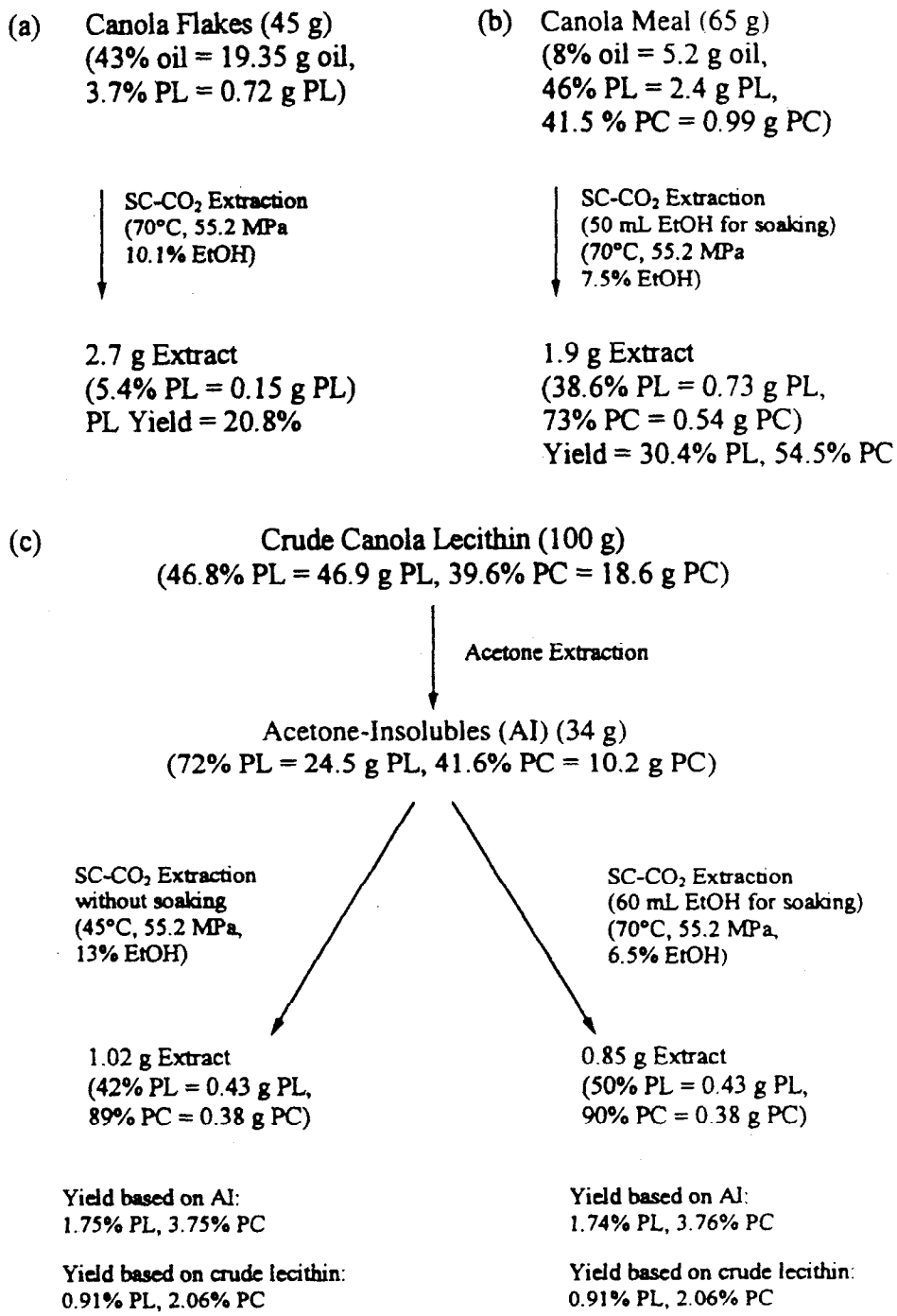


Fig. 13.2. Flow schematic for SC-CO_2 phospholipid recovery from canola flakes (PL = phospholipids; PC = phosphatidylcholine). *Source:* Dunford, T.G., and F. Temelli, *J. Amer. Oil Chem. Soc. 72*:1009 (1995).

extract the residual phospholipid content of the meal using extraction conditions of 68.3 MPa and 80°C and varying the ethanol content of the supercritical fluid phase from 5 to 20 mol%. They confirmed what others have found, that there was an increase in the phospholipid content of the extract, which could be optimized at a 15 mol% addition of ethanol to the SC-CO_2. Extensive analysis of the phospholipid-laden fractions by Bollman's reagent, phosphorus analysis by inductively coupled plasma spectroscopy, and high-performance liquid chromatography showed that phosphatidylcholine and phosphatidylethanolamine could be recovered at levels exceeding 90% from the deoiled meal. The more polar phospholipids in the soybean flakes could also be obtained at 70% recoveries using 20 mol% ethanol in SC-CO_2. These high recoveries could only be obtained by using both a cosolvent and the higher extraction pressures and temperatures quoted above.

Conclusions

This chapter has shown that many attributes can be imparted to the residue, such as seed meals, by extraction with supercritical fluids. The obvious absence of objectionable solvent residues in the case of processing with CO_2 is very attractive and applies equally to both extractable and nonextractable components. We have also seen how treatment with SC-CO_2 can produce an unaltered residual, equivalent to those obtained via other processing techniques. On the other hand, examples have been cited where treatment with SC-CO_2 can alter enzyme activity, thereby affecting the end use properties of the extracted substrate.

Substrates remaining after partial or exhaustive extraction can also be reextracted with SC-CO_2 and an appropriate cosolvent. This often results in the recovery of a high-value product that can be utilized in a variety of ways while retaining the nutritive value of extracted product. As researchers become even more facile in the application of pressure and supercritical fluids, new substrate morphologies may be generated that have different chemical as well as dissolution properties, thereby opening up other application opportunities for these supercritical fluid–processed materials.

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